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Safety and efficacy of PfSPZ Vaccine against *Plasmodium falciparum* via direct venous inoculation in healthy malaria-exposed adults in Mali: a randomised, double-blind phase 1 trial

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MSS, MW, and SAH were the principal investigators. MSS, SAH, MW, FO, MPF, IZ, SLH, OD, and PED designed the trial with contributions from all authors in the review of the approved final version and additional amendments. MSS, AK, BK, YS, MAG, AD, AN, KN, AZ, KS, HD, IT, and KD collected the data. IZ, MAG, KN, HD, EMO'C, TBN, ML, AM, and SC completed the study laboratory endpoints. BKLS, PFB, ERJ, and SLH developed the vaccine. AD, AN, KN, AZ, YA, BKLS, PFB, and ERJ completed the procedures and syringe preparations for injection. SW-M, TM, and AG provided regulatory and project support during the course of the study. EEG, MPF, and AJR completed the statistical analysis. MSS, SAH, IZ, EEG, MPF, AJR, SC, TLR, SLH, OD, and PED interpreted data and results.

See Online for appendix

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Summary

Background—*Plasmodium falciparum* sporozite (PfSPZ) Vaccine is a metabolically active, non-replicating, whole malaria sporozoite vaccine that has been reported to be safe and protective against *P falciparum* controlled human malaria infection in malaria-naive individuals. We aimed to assess the safety and protective efficacy of PfSPZ Vaccine against naturally acquired *P falciparum* in malaria-experienced adults in Mali.

Methods—After an open-label dose-escalation study in a pilot safety cohort, we did a double-blind, randomised, placebo-controlled trial based in Donéguébougou and surrounding villages in Mali. We recruited 18–35-year-old healthy adults who were randomly assigned (1:1) in a double-blind manner, with stratification by village and block randomisation, to receive either five doses of $2{\cdot}7 \times 10^5$ PfSPZ or normal saline at days 0, 28, 56, 84, and 140 during the dry season (January to July inclusive). Participants and investigators were masked to group assignments, which were unmasked at the final study visit, 6 months after receipt of the last vaccination. Participants received combined artemether and lumefantrine (four tablets, each containing 20 mg artemether and 120 mg lumefantrine, given twice per day over 3 days for a total of six doses) to eliminate *P falciparum* before the first and last vaccinations. We collected blood smears every 2 weeks and during any illness for 24 weeks after the fifth vaccination. The primary outcome was the safety and tolerability of the vaccine, assessed as local and systemic reactogenicity and adverse events. The sample size was calculated for the exploratory efficacy endpoint of time to first *P falciparum* infection beginning 28 days after the fifth vaccination. The safety analysis included all participants

who received at least one dose of investigational product, whereas the efficacy analyses included only participants who received all five vaccinations. This trial is registered at ClinicalTrials.gov, number .

Findings—Between Jan 18 and Feb 24, 2014, we enrolled 93 participants into the main study cohort with 46 participants assigned PfSPZ Vaccine and 47 assigned placebo, all of whom were evaluable for safety. We detected no significant differences in local or systemic adverse events or laboratory abnormalities between the PfSPZ Vaccine and placebo groups, and only grade 1 (mild) local or systemic adverse events occurred in both groups. The most common solicited systemic adverse event in the vaccine and placebo groups was headache (three [7%] people in the vaccine group *vs* four [9%] in the placebo group) followed by fatigue (one [2%] person in the placebo group), fever (one [2%] person in the placebo group), and myalgia (one [2%] person in each group). The exploratory efficacy analysis included 41 participants from the vaccine group and 40 from the placebo group. Of these participants, 37 (93%) from the placebo group and 27 (66%) from the vaccine group developed *P falciparum* infection. The hazard ratio for vaccine efficacy was 0·517 (95% CI 0·313–0·856) by time-to-infection analysis (log-rank p=0·01), and 0·712 (0·528–0·918) by proportional analysis (p=0·006).

Interpretation—PfSPZ Vaccine was well tolerated and safe. PfSPZ Vaccine showed significant protection in African adults against *P falciparum* infection throughout an entire malaria season.

Introduction

The fight against malaria has intensified, with major funding organisations now pursuing its eradication. Yet despite at least US$2·5 billion of investment, in 2015 there were 214 million malaria cases and 438 000 deaths, mostly due to *Plasmodium falciparum.*[1] A highly effective vaccine is urgently needed to control and eradicate malaria.

A vaccine for use in mass campaigns to eliminate *P falciparum* will have to be well tolerated and safe to ensure compliance, and must be highly protective. So far, only *P falciparum* sporozites (PfSPZ) have induced sterile immunity against controlled human malaria infection (CHMI) in more than 90% of recipients. This finding was originally observed in volunteers immunised by the bites of more than 1000 mosquitoes carrying radiation-attenuated PfSPZ.[2–5] Manufacturing advances have now allowed the production of aseptic, purified, cryopreserved PfSPZ from the NF54 isolate, which is suitable for human use.[6,7] In a dose-escalation trial, six malaria-naive participants in the USA who received five intravenous doses of $1{\cdot}35 \times 10^5$ PfSPZ Vaccine (Sanaria, Rockville, MD, USA) showed high-level, short-term protection against homologous CHMI.[8] Results from subsequent studies in malaria-naive individuals have shown a high level of protective efficacy against homologous CHMI with as few as three doses of PfSPZ Vaccine and evidence of durable long-term protection.[9,10]

In this study, we aimed to assess the tolerability, safety, immunogenicity, and protective efficacy of PfSPZ Vaccine in healthy, malaria-experienced adults living in an area of Mali, west Africa, which has seasonally intense *P falciparum* transmission.

Methods

Study design and participants

We did this double-blind, randomised, placebo-controlled phase 1 trial in Doneguebougou, Mali, and surrounding villages (four villages in total), with the study being done at a single centre. Doneguebougou is a rural community about 30 km north of Bamako, the capital of Mali. Malaria transmission usually occurs from July through to the end of December.[11]

Participants were eligible to enrol if they were healthy adult (18–35 years old) men or non-pregnant women, provided informed consent, and had resided at the study site for at least the past 4 years. Women of child-bearing potential who wished to participate had to be willing to use reliable contraception for the duration of the vaccination phase of the study. People were excluded from participation if they had known allergies or contraindications to any of the study interventions (PfSPZ Vaccine or artemether and lumefantrine), had previously received a malaria vaccine, had abnormal laboratory findings, or had known recent use of antimalarial medications, investigational products, immunosuppressive medications, or blood products. A history of a serious chronic illness, known alcohol or drug misuse, any clinically significant abnormalities on a 12 lead electrocardiogram, and a positive tests for HIV, hepatitis B, hepatitis C, syphilis, or sickle cell disease or trait were also exclusion criteria. A full list of inclusion and exclusion criteria is available in the appendix (pp 4, 5).

The trial was done in accordance with the provision of the Good Clinical Practice guidelines and in alignment with institutional procedures and guidelines. Each participating village provided community permission and all participants provided written informed consent.[12] The study was approved by the ethics review board in Mali (Faculté de Médecine de Pharmacie et d'OdontoStomatologie [FMPOS], Bamako, Mali), the US National Institute of Allergy and Infectious Diseases (NIAID; National Institutes of Health [NIH], Bethesda, MD, USA) institutional review board, Mali national regulatory authority, and was conducted under FDA IND 14826.

Randomisation and masking

For safety, we did the trial in a stepwise manner with two cohorts: the pilot safety cohort and the main cohort. For the pilot safety cohort, the first eligible participants available were enrolled in an unmasked fashion. Both participants and clinical staff were aware of the PfSPZ Vaccine being given in this cohort. In the main cohort, participants were stratified by village and block randomised in a double-blind manner. Participants were randomly assigned (1:1) to receive five doses of either $2{\cdot}7 \times 10^5$ PfSPZ Vaccine or placebo (normal saline). Participants in the main cohort and investigators were masked to these group assignments. To maintain masking, the randomisation code was provided directly by the study statistician to the site pharmacist via secure email before the vaccinations started. The product provided to the clinic for injections was labelled only with the participant's study identification number, and the volume (0·5 mL) and colour of the vaccine and placebo injections were identical. Group assignments were unmasked at the final study visit, which occurred 6 months after receipt of the fifth vaccination.

Procedures

PfSPZ Vaccine contained aseptic, purified, cryopreserved PfSPZ manufactured as described previously.[6–8] Sterile isotonic normal saline (Hospira, Lake Forest, IL, USA), identical in appearance to PfSPZ Vaccine, was procured in the USA. 0·5 mL of vaccine or placebo was injected into an arm vein by direct venous inoculation through a 25 gauge needle over the course of several seconds. PfSPZ Vaccine was injected within 30 min of thawing. Vaccinations were administered at the study centre in Donéguébougou, Mali. Local physicians trained in the procedure, but who were not involved in participant follow-up or adverse event assessment, did the injections. Participants in the pilot safety cohort received two open-label doses of PfSPZ Vaccine: $1{\cdot}35 \times 10^5$ on day 0 and $2{\cdot}7 \times 10^5$ on day 14. Participants in the main cohort received doses of $2{\cdot}7 \times 10^5$ PfSPZ Vaccine (total dose $13{\cdot}5 \times 10^5$ PfSPZ) or normal saline placebo on days 0, 28, 56, 84, and 140 during the dry season (January to July inclusive; appendix p 12). Participants in the pilot safety cohort had the option to join the main cohort and receive three additional doses of $2{\cdot}7 \times 10^5$ PfSPZ Vaccine, for a total dose of $12{\cdot}15 \times 10^5$ PfSPZ Vaccine. After the last vaccination, participants were actively followed up during the transmission season for 24 weeks.

After each vaccination, participants were monitored for at least 2 h for local and systemic adverse events. Participants were assessed on site for safety on days 1, 3, and 7 after vaccination, and medically qualified study personnel were available at all times for unscheduled visits. Solicited local and systemic adverse events were recorded for 7 days after vaccination (appendix p 14). Unsolicited adverse events, including symptomatic malaria, serious adverse events, and new chronic medical conditions were recorded throughout the study. Our definition of serious adverse events is available in the appendix (p 6). Protocol-specified laboratory assessments, including complete blood count with differential, creatinine, and alanine aminotransferase were completed after each vaccination. Blood was drawn for protocol-specified laboratory assessments prior to each vaccination and on days 3 and 7 after each vaccination. Grading of adverse events was based on the US Food and Drug Administration's (FDA) guidelines for vaccine clinical trials[13] and adapted to local normal reference ranges (appendix p 15).

We deemed participants to be enrolled once they had received a directly observed full treatment course of combined artemether and lumefantrine (Novartis, Basel, Switzerland) roughly 1–2 weeks before first vaccination. The course consisted of artemether and lumefantrine combination tablets containing 20 mg artemether and 120 mg lumefantrine, with four tablets given twice per day over 3 days for a total of six doses. Participants received another course of artemether and lumefantrine roughly 3 weeks before their final vaccination.

We assessed potential co-infections at a single time point before the fifth vaccination. Gastrointestinal helminths and protozoa were detected in stool samples by a modified qPCR technique (appendix pp 9, 18) and *Schistosoma haematobium* eggs were quantified in real time by microscopy after filtration of fresh urine samples and staining with 5% ninhydrin.[14] Stool samples were aliquoted with absolute ethanol and cryopreserved at −80°C in Mali and then shipped to the USA on dry ice for analysis by the Laboratory of Parasitic Diseases at NIAID/NIH, whereas urine samples were analysed at the study centre by the College of

American Pathologists (CAP) certified Malaria Research and Training Centre (MRTC) clinical laboratory.

Thick blood smears were prepared before each vaccination or when clinically indicated during the vaccination period (appendix p 6). Starting 2 weeks after the last vaccination, blood smears were examined every 14 days, or during suspected malaria illness. Symptomatic malaria was defined as *P falciparum* asexual parasitaemia accompanied by an axillary temperature of at least 37·5°C, clinical signs and symptoms of malaria, or both. Antimalarial treatment with the standard treatment course of artemether and lumefantrine was provided for symptomatic malaria; asymptomatic parasitaemia was not treated, in accordance with the treatment guidelines of the Malian Government. Blood smears were examined in accordance with standard procedures by technicians with documented training and experience in slide reading.

We measured antibodies against PfSPZ by use of an automated immunofluorescence assay and antibodies against recombinant *P falciparum* circumsporozoite protein (PfCSP) via an ELISA (appendix pp 6–9). For biologically-active antibodies against PfSPZ, we used an inhibition of sporozoite invasion assay with human hepatocyte line (HC-04) in the presence of post-immunisation versus pre-immunisation sera from the same individual (appendix p 8). Using ELISA, we assessed antibodies to proteins first expressed in sporozoites (PfSSP2/TRAP, PfMSP5, PfAMA1, PfCelTOS), early liver stage parasites (PfLSA1, PfEXP1), and late liver stage parasites (PfMSP1, PfEBA175) before immunisation and 2 weeks after last vaccine. We assessed vaccine-induced ex-vivo T-cell responses with multi-parameter flow cytometry on fresh peripheral blood mononuclear cells. Whole blood was stained ex vivo to measure levels of CD4, CD8, and γδ T cells, and NK cells, before vaccination and after vaccination (days 3, 7 and 27 after each vaccination plus day 14 post-dose 5).

Outcomes

The primary aim of the study was to assess the safety of repeated immunisation by direct venous inoculation with PfSPZ Vaccine in adults in Mali. We assessed this outcome as the incidence and severity of local and systemic adverse events occurring within 7 days after each vaccination. Protective efficacy against naturally occurring *P falciparum* infection (positive thick blood smears) and symptomatic malaria (defined as *P falciparum* asexual parasitaemia accompanied by an axillary temperature of at least 37·5°C, clinical signs and symptoms compatible with malaria, or both) were exploratory endpoints of this phase 1 study. We defined positive blood smears as detection of at least two *P falciparum* parasites by microscopic examination of 0·5 μL of blood during malaria transmission season, starting at study week 28 (4 weeks after the last vaccination; primary exploratory efficacy endpoint) through to the end of the study or starting at the time of the last vaccination (secondary exploratory efficacy endpoint) through to the end of the study. Phenotypic and functional characterisation of humoral and cellular immune responses were also exploratory endpoints, and we planned to characterise and compare host immune responses to vaccination and to specific *P falciparum* antigens (PfSPZ, CSP, MSP1, SSP2, MSP5, AMA1, EXP1, LSA1, EBA175, CelTOS) in adults in Africa. Samples collected before or after immunisation with PfSPZ Vaccine were assessed by various humoral and cellular assays, described in the

appendix (pp 6–9), and we analysed the immune responses for differences between people who received vaccine and those who received placebo, and between vaccine recipients who became infected with malaria or those who remained uninfected throughout follow-up.

Statistical analysis

All participants who received at least one dose of investigational product were included in safety analyses, including those in the pilot safety cohort, whereas only participants who received all five vaccinations were included in the efficacy analyses. The prespecified primary exploratory efficacy outcome was time to first positive blood smear 28 days or more after the final vaccination. On the basis of historical unpublished data about malaria infection in adults in Donéguébougou, we decided before the start of the study that it was reasonable to assume that at least 50–65% of individuals in the control group would develop parasitaemia during the 20 week observation period. Assuming an exponential model and using a two sided log-rank test at the 5% level, a sample size of 45 participants per group would give more than 80% power to detect a vaccine efficacy (based on incidence rates) of 60% if 65% of individuals in the control group developed parasitaemia.

Our main test for differences in time to first positive blood smear was an interval-censored log-rank test, because of interval censoring of data, performed using the R package interval with vaccine efficacy quantified by the Cox proportional hazards model.[15] We did the proportional analysis on the binary endpoint of any positive blood smears from 28 days after fifth vaccination through to the end of the malaria season by a conditional exact test with melded confidence intervals, using R package exact2×2.[16] We also did efficacy analyses for the time-to-event and proportional endpoints on data starting from the day of the fifth vaccination (main cohort only). Most analyses were per-protocol, meaning that only participants in the main cohort who completed all five vaccinations were included. We also did a sensitivity analysis of the proportional secondary endpoint to investigate the intention-to-treat results (appendix p 10).

We did all statistical analyses with R version 3.3.1. The study was monitored for safety by an independent Data and Safety Monitoring Board and a local medical monitor. This trial is registered at ClinicalTrials.gov, number .

Role of the funding source

The funders were involved in the study design, study management, data collection, data analysis, data interpretation, and writing of the report. The principal investigators (MSS, SAH) had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Participants were enrolled in the study from Jan 18, 2014, to Feb 24, 2014, and the last vaccinations were given between July 14, 2014, and July 17, 2014. 12 people enrolled into the pilot safety cohort (figure 1) and received their first and second vaccinations (appendix p 12) before the start of the main cohort. Nine members of the pilot safety cohort joined the main cohort to receive an additional three vaccinations. 97 participants enrolled into the

main cohort (figure 1), but one was excluded for non-compliance before randomisation. Of the 96 participants who entered randomisation, 48 were allocated to receive PfSPZ Vaccine and 48 to receive placebo. Two participants in the vaccine group and one participant in the placebo group were excluded before any vaccinations. 46 participants in the vaccine group and 47 participants in the placebo group received at least one vaccination and were eligible for inclusion in the safety analyses. 88 participants (44 in the vaccine group and 44 in the placebo group) received all five vaccinations starting in the week of July 14, 2014 (appendix p 12). Of these 88 people, 86 (42 in the vaccine group and 44 in the placebo group) completed follow-up through to the last study visit (figure 1). Baseline characteristics seemed to be well balanced between the vaccine and placebo groups (table 1), with most participants being young (mean age 25 years [SD 5]) and male (90 [86%] of 105 participants).

Of the main cohort who received at least one vaccination, six (13%) of 46 participants in the vaccine group and five (11%) of 47 participants in the placebo group were blood-smear positive for *P falciparum* at the time of screening (table 1). All 93 participants completed treatment with artemether and lumefantrine treatment, with the last dose given to the vaccine group a mean of 3·6 days (SD 1·7) before the first vaccination and to placebo group a mean of 4·0 days (1·6) before. Subsequent to drug treatment, all blood smears were negative for *Plasmodium* species throughout the 20 weeks of vaccinations. All 88 participants remaining in the main cohort received artemether and lumefantrine before their last vaccination, with the last drug dose given to the vaccine group a mean 20·8 days (SD 0·9) before the last vaccination and to placebo group 20·9 days (0·8) before.

We assessed co-infections with other pathogens in 80 people in the main cohort (40 in the vaccine group and 40 in the placebo group) and all nine participants who received five vaccinations in the pilot safety cohort before their fifth vaccination. Few co-infections were detected, with no substantial differences between vaccine and placebo groups (table 1), and no associations with *P falciparum* infection during follow-up.

502 injections were given, generally each in less than 10 s. Only one missed injection needed repeat administration, which occurred during the first vaccinations in the pilot safety cohort. Vaccinations were well tolerated and safe, with no serious adverse events. Most study participants reported no local or systemic adverse events after vaccination (table 2). Only grade 1 (mild) local or systemic adverse events were reported. None of the vaccine group participants and four (9%) of the 47 placebo group participants reported local injection site pain. Overall, three (7%) people in the vaccine group and four (9%) people in the placebo group reported any systemic adverse events after vaccination (table 2). The most common solicited systemic adverse event in the vaccine and placebo groups was headache (three [7%] people in the vaccine group *vs* four [9%] in the placebo group) followed by fatigue (one [2%] person in the placebo group), fever (one [2%] person in the placebo group), and myalgia (one [2%] person in each group; table 2). Local or systemic adverse events did not differ significantly between the vaccine and placebo groups (all p values >0·15). Laboratory abnormalities did not differ between the vaccine and placebo groups (appendix p 22) and did not increase with successive vaccinations.

Our analysis of occurrence and time to first *P falciparum* infection during a 20 week period up to the end of the malaria season, starting 28 days after the fifth vaccination (starting the week of Aug 11, 2014) included 81 participants (41 in the vaccine group and 40 in the placebo group; figure 2). In the placebo group, 37 (93%) of 40 participants became blood-smear positive by the end of the study period compared with 27 (66%) of 41 participants in the vaccine group. Vaccine recipients had a significantly lower hazard of *P falciparum* infection, with a Cox hazard ratio (HR) for vaccine efficacy of 0·517 (95% CI 0·313–0·855; log-rank p=0·01). Additionally, the proportion of participants with any infection from 28 days after the fifth vaccination to the end of the malaria season was lower in the vaccine group than in the control group (HR 0·712, 0·528–0·918; p=0·006).

For vaccine efficacy analysed during 24 week period starting immediately after the fifth vaccination, 86 participants (42 in the vaccine group and 44 in the placebo group) were evaluable for the proportional analysis and 87 (43 in the vaccine group and 44 in the placebo group) participants were evaluable for the time to infection analysis; one person in the vaccine group and four people in the placebo group had a positive blood smear on day 14 after last dose. The results for vaccine efficacy based on the Cox HR for time to infection (HR 0·479, 95% CI 0·294–0·781; log-rank p=0·005) and proportional analysis (0·715, 95% CI 0·536–0·910; p=0.004) were similar to those of the primary exploratory analyses (appendix p 20). The results of the opposite-arm imputation sensitivity analysis supported the per-protocol findings for the proportional analysis (p=0·02; appendix p 10).

Incidence of first infections plateaued in the vaccine group at 14 weeks after the last vaccination, after which only two (13%) of 16 participants in vaccine group but five (63%) of eight participants in the placebo group who had previously been uninfected had their first infection. During the same 10 week period, 16 previously infected and treated individuals (eight in the vaccine group and eight in the placebo group) had repeat infections, indicating that malaria transmission continued throughout follow-up.

Among the 88 participants (44 in the vaccine group and 44 in the placebo group) in the main cohort who received all five vaccinations, 42 people (20 in the vaccine group and 22 in the placebo group) were treated for symptomatic malaria at least once. Overall, risk of symptomatic malaria did not significantly differ between the vaccine and placebo groups (HR 0·91, 95% CI 0·50–1·67, p=0·77), although the study was not designed to show such a difference.

At 2 weeks after fifth vaccination, antibody responses to PfCSP (assessed with ELISA) and PfSPZ (assessed with automated immunofluorescence assay) were significantly larger in the vaccine group than in the placebo group, although the differences were small (p<0–0001 by the Wilcoxon rank sum test; figure 3A–C). However, functional antibodies to PfSPZ (assessed by inhibition of sporozoite invasion) did not differ significantly between groups. We detected no significant difference between infected and uninfected vaccinated individuals in terms of antibodies to PfSPZ (logistic regression odds ratio [OR] 1·00, 95% CI 1·00–1·00, p=0·934; figure 3A), percentage inhibition of PfSPZ invasion (logistic regression OR 0·99, 0·98–1·01, p=0·288; figure 3B), or the net change in PfCSP antibody response after the fifth vaccination (logistic regression OR 0·77, 1·05–0·57, p=0·096; figure

3C). Nine (64%) of 14 vaccine recipients who remained uninfected versus nine (32%) of 28 vaccine recipients who became infected had an arbitrarily defined increase in antibodies to PfCSP. The logistic regression for log fold rise in PfCSP antibodies gave an OR of 0·53 (95% CI 0·26–1·1; p=0·07; figure 3D) for uninfected people versus infected people in the vaccine group. Time to first infection was significantly associated with a fold rise in PfCSP antibody levels from before vaccination to 2 weeks after the fifth immunisation in the vaccine group (HR log fold rise in vaccine group 0·57, 0·40–0·81, p=0·002; figure 3F), but not in the placebo group (0·87, 95% CI 0·71–1·1, p=0·18; figure 3E). Infected and uninfected individuals in the vaccine group did not differ in terms of antibodies measured in automated immunofluorescence assays or inhibition of sporozoite invasion tests (figure 3A, B).

Antibody responses to protein first expressed in sporozoites (PfSSP2/TRAP, PfMSP5, PfAMA1, PfCelTOS), early liver stage parasites (PfLSA1, PfEXP1), and late liver stage parasites (PfMSP1, PfEBA175) were low and did not correlate with infection; the maximum seroconversion rate in any group to any of these antigens was 14% (appendix p 24). In ex-vivo samples, whole blood CD4, CD8, and γδ T cells and NK cells did not differ between the vaccine and placebo groups at any time point (appendix pp 25, 26).

Discussion

PfSPZ Vaccine given via direct venous inoculation to healthy adults in Mali was safe, well tolerated, and protective. To our knowledge, our study is the first trial of a whole malaria sporozoite vaccine in the field. In this study, the placebo control was normal saline, yet solicited and unsolicited adverse events and laboratory abnormalities did not differ between the vaccine and placebo groups, which is unusual for a vaccine trial, but consistent with the results of other clinical trials of PfSPZ Vaccine.[7–10,17] In these studies, aseptic, purified, cryopreserved whole PfSPZ have shown an excellent safety and tolerability profile.

To our knowledge, the protective efficacies of about 48% by time to first positive blood smears and about 29% by proportion of participants with at least one positive blood smears during a full malaria transmission season (20 weeks), are higher than those reported for other malaria vaccine candidates. However, comparisons of vaccine efficacies between trials are limited by differences in trial designs, including sample sizes, endpoints, and statistical approaches. For example, a viral vectored vaccine given to adults in Kenya in a low transmission setting conferred 67% efficacy against PCR-detected *P falciparum* during an 8 week period,[18] but none of these individuals were treated and none progressed to positive blood smears, making it difficult to compare that trial to our current trial or to other field trials for which blood smear positivity is the standard endpoint. The PfCSP vaccine, RTS,S, conferred 34% efficacy by time to first positive blood smears in adults in The Gambia when formulated with AS02 adjuvant, but no significant protection by proportion of participants with at least one positive blood smear during the 15 weeks of follow-up.[19] In a trial powered to detect 45% vaccine efficacy, vaccination of Kenyan adults with RTS,S formulated with $AS01_B$ or $AS02_a$ adjuvants did not achieve significant protection against positive blood smears during 16 weeks of follow-up.[20]

In our study in Mali, protective efficacy was achieved despite intense transmission of heterologous African *P falciparum* parasites, with 93% of the placebo group being infected during follow-up. This intense transmission exceeds that reported in previous trials of malaria vaccines in adults in Africa.[18–21] Protective efficacy was sustained throughout follow up, as shown by the inverse survival curve in figure 2, which remained flat during the last 10 weeks. During this time, seven new infections and 16 re-infections occurred in the study cohort as a whole, showing ongoing transmission. Sustained efficacy against infection during continued transmission has, to our knowledge, not been seen in trials of other malaria vaccines in Africa.

Although not defined endpoints of the study, the number of symptomatic malaria occurrences, the number of unique participants with symptomatic malaria, and the time to first symptomatic malaria event did not significantly differ between the placebo and vaccine groups (appendix p 21, 23). In those participants with a previous or concurrent positive blood smear in either the vaccine or placebo groups, the time to, incidence of, and severity of the first symptomatic malaria episode were also similar (appendix p 21). These results are not surprising because the immunity induced by PfSPZ Vaccine is active against the pre-erythrocytic rather than erythrocytic stages of malaria, and would not be expected to directly reduce parasite densities in the blood. Nevertheless, we could speculate that people with pre-existing partial immunity might achieve even higher levels of protection from symptomatic malaria after receiving a vaccine that prevents infection in roughly 30% individuals as a result of reductions in the size and frequency of merozoite inocula into the blood. Although our study was not powered to examine symptomatic malaria as an endpoint, our data do not support this hypothesis. However, a vaccine that prevents infection in a high proportion of recipients will be expected to significantly reduce the number of individuals with symptomatic malaria as well.

Our goal is to develop a vaccine that can be used in mass vaccination programmes to eliminate *P falciparum* from geographically defined areas. To be useful for this indication, the vaccine will have to prevent infection in at least 80% of recipients for roughly 24 weeks by some estimates.[17,22] The protective efficacy of about 29% shown in our proportional analysis through 24 weeks of follow-up, while significant, is not adequate to achieve this objective. Furthermore, this level of efficacy is lower than the 64% protection seen in malaria-naive individuals from the USA who received the same PfSPZ Vaccine regimen and underwent CHMI with homologous NF54 parasites, although it is higher than the 8% protection in those who underwent CHMI with heterologous 7G8 parasites 24 weeks after their last vaccine dose.[10] Notably, net change in antibodies to PfCSP at 2 weeks after the fifth dose was substantially lower in people in Mali (median 393, IQR 11–2242) than in the participants in the USA (12 047, 2615–19 373) who received the same immunisation regimen, which might show that cellular responses in the liver—believed to be the main mechanism of protective immunity induced by PfSPZ Vaccine—were also lower. Additionally, the median pre-vaccination serum dilution in Mali was 535 (IQR 314–981), compared with 46 (26–84) in the USA, suggesting that the immunisations were administered against a higher background of naturally acquired immunity in Mali. Helminth infections have been associated with poor vaccine responses,[23,24] but were uncommon in our study. On the basis of previous studies in animals[25,26] and in the field,[27] we suggest that the poor

immunogenicity and less than optimal protective efficacy of PfSPZ Vaccine in our study were caused by immunoregulation based on lifelong exposure to *P falciparum* and a sub-optimal immunisation regimen, both of which could be overcome by increasing numbers of PfSPZ per dose and varying the timing and numbers of doses. Several studies testing this hypothesis are now underway in Africa, the USA, and Europe. A trial ongoing in Mali () is examining whether a three dose regimen (total dose $5{\cdot}4 \times 10^6$ PfSPZ *vs* $1{\cdot}4 \times 10^6$ PfSPZ for our current trial) can confer higher protective efficacy in the same population by use of a more practical but higher dose regimen. Additionally, parasite diversity in the study area might contribute to the suboptimal efficacy seen in Mali, and whole-genome sequencing studies of parasites collected from this trial are in process to examine this question.

Most data from animal studies with rodent *(Plasmodium berghei* and *Plasmodium yoelii)* and simian *(Plasmodium knowlesi)* parasites suggest that cellular immune responses against the parasite in hepatocytes are responsible for the protective immunity induced by immunisation with attenuated sporozoites and that assessment of cellular immune responses in peripheral blood does not reflect the status of cellular immunity in the liver.[7,8,28,29] Given that antibody responses against PfCSP and PfSPZ were lower in Mali compared with participants in the USA, and therefore unlikely to prevent invasion of all sporozoites into hepatocytes, our results are consistent with this perspective. However, even with the lower antibody responses, anti-PfCSP antibodies were associated with infection status, as seen in a US study.[8] As stated previously, anti-PfCSP antibodies in the blood might be predictive of protective cellular immune responses occurring in the liver.[7]

Our trial has important limitations. We exclusively enrolled healthy adults in an area with intense seasonal *P falciparum* transmission, and additional studies are needed to assess the safety, feasibility, and efficacy of PfSPZ Vaccine in other age groups, study populations (eg, during pregnancy or in immunocompromised individuals), and transmission settings, especially in view of the proposed indication for elimination. One concern about the widespread use of PfSPZ Vaccine has been feasibility of administration by direct venous inoculation, which is not used for any licensed preventive vaccines against infectious diseases. In this trial, 491 consecutive inoculations with 0·5 mL injections were completed on first attempt and were well tolerated; the process took only seconds from the time of the introduction of the needle to the completion of the injection. This successful implementation of direct venous inoculation in adults in a rural, malaria-endemic setting is a substantial advance. Studies are now underway to assess direct venous inoculation in adolescents, children, and infants. Another concern has been the logistical implementation of a malaria vaccine that requires liquid nitrogen for storage and transport. We did not find the logistics of PfSPZ Vaccine shipment, storage, and distribution to the field sites to be more burdensome than those of other refrigerated vaccines we have tested in the field. Notably, the handling, maintenance, and transport of the dry shipper holding the PfSPZ Vaccine were independent of electricity and required little maintenance. A reliable liquid nitrogen cold chain distribution network is needed for PfSPZ Vaccine, and might require new investment in some communities, but initial analyses suggest that the costs could be competitive with standard distribution models currently in place for 2–8°C vaccines.[30]

Our long term goal is to develop a PfSPZ Vaccine regimen that meets the requirements for mass administration and elimination of *P falciparum* from geographically defined areas. To be suitable for this goal, the regimen must be well tolerated and safe, easy to distribute and to administer, and highly protective. Our data suggest that PfSPZ Vaccine at the doses tested is well tolerated and safe and can be given reliably to healthy adults in the field in Africa. Clinical trials are now underway on three continents to optimise the regimen to achieve higher levels of protective efficacy, and to study this vaccine in other demographic groups.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Declaration of interests

TM, AJR, ML, YA, AM, AG, SC, BKLS, PFB, ERJ, TLR, and SLH are salaried, full-time employees of Sanaria, the developer and sponsor of Sanaria PfSPZ Vaccine. FO was employed by Sanaria and received financial support from Sanaria for travel. SLH and BKLS also have financial interests in Sanaria. ML, SC, BKLS, and SLH are inventors on patents and applications for patent that have been assigned to Sanaria. All other authors declare no competing interests.

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Research in context

Evidence before this study

We searched PubMed, the Cochrane Library, Google Scholar, Scopus, and Web of Science on Oct 30, 2016, for English-language articles on randomised controlled trials of malaria vaccines in adults published between Jan 1, 1980, and Oct 30, 2016. We searched using the terms (“malaria vaccines” [MeSH Terms] OR “malaria” [All Fields] AND “vaccines” [All Fields]) OR “malaria vaccines” [All Fields] OR (“malaria” [All Fields] AND “vaccine” [All Fields]) OR “malaria vaccine” [All Fields]) AND (PfSPZ [All Fields] AND PfSPZ Vaccine [All Fields]) AND (“adults” [MeSH Terms] OR “adults” [All Fields]). For the Cochrane Library and other data sources, we used the key search terms “PfSPZ”, “malaria vaccines”, “adults”, AND “clinical trials”. We did not identify any previous studies that examined the efficacy of a whole malaria sporozoite vaccine in a malaria-endemic population.

Added value of this study

Although the protective efficacy of *P falciparum* sporozites (PfSPZ) Vaccine against controlled human malaria infection has been assessed many times, to our knowledge this is the first report of the protective efficacy of PfSPZ Vaccine against malaria in the field, the largest PfSPZ Vaccine trial reported so far, and the first trial of any whole malaria sporozoite vaccine to show some protection against natural infection. PfSPZ Vaccine was easy to administer by direct venous inoculation of healthy adults, and was well tolerated and safe. However, the anti-P *falciparum* circumsporozoite protein antibody response was substantially lower in Malians than has previously been reported in healthy US adults.

Implications of all the available evidence

The findings from our study in Mali lay the foundations for further assessment of PfSPZ Vaccine across the USA, Europe, and Africa, which will help to finalise regimens for phase 3 clinical trials and assess this candidate product in other demographic groups. We have shown that PfSPZ Vaccine is safe and well tolerated and can confer sustained protective efficacy to healthy adults during an entire malaria transmission season in an area with seasonally intense *P falciparum* transmission.

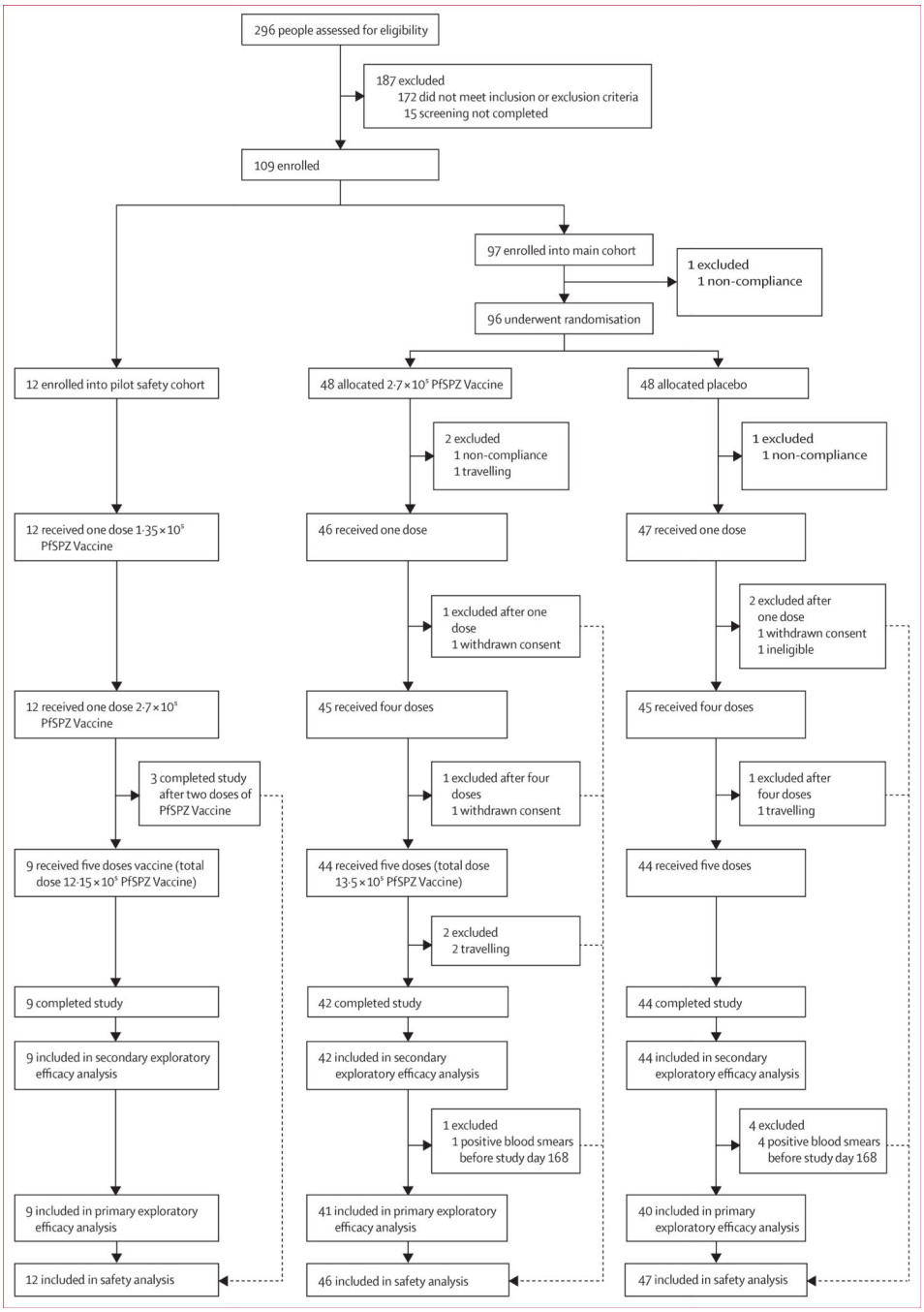

Figure 1: Trial profile

Study completion was defined as staying in the study until the end of malaria transmission season (study day 308). PfSPZ=*Plamodium falciparum* sporozite.

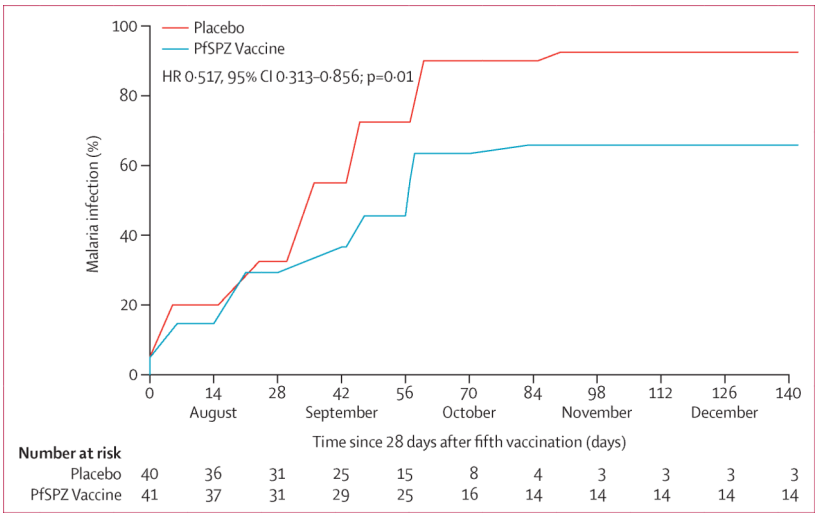

Figure 2: Protective efficacy of PfSPZ Vaccine against naturally occurring infection

Protective efficacy was analysed by time to first positive blood smear, with day 0 at 28 days after the fifth vaccination. The inverse survival curves include participants who received all five vaccinations and were evaluable for the primary exploratory efficacy endpoint. Five participants (one in the PfSPZ Vaccine group and four in the placebo group) were censored from the primary efficacy analysis because they had a positive blood smear before 28 days after the fifth vaccination. PfSPZ=*Plamodium falciparum* sporozite.

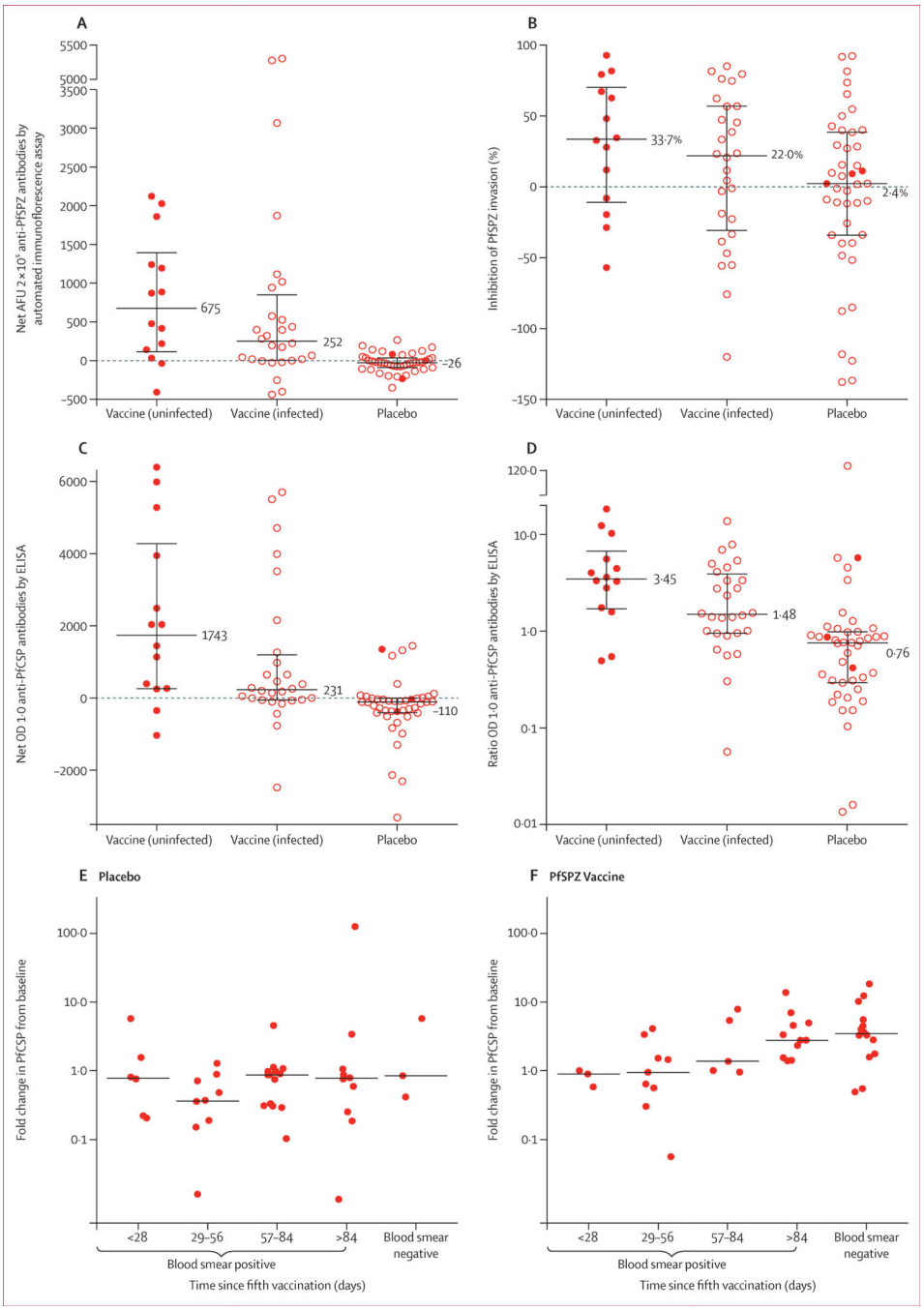

Figure 3: Antibody responses

Antibody responses to PfSPZ and PfCSP were measured before immunisation and at 2 weeks after the fifth dose of PfSPZ Vaccine. For all assays, uninfected individuals are shown as filled (red) circles and infected individuals are unfilled circles. For each of the defined groups, the median response values are shown with bars representing the IQR. (A) Antibodies to PfSPZ by automated immunofluorescence assay are reported as net AFU 2×10^5; the reciprocal serum dilution at which the fluorescent units were 2×10^5 in post-immunisation minus pre-immunisation sera. (B) Percentage inhibition of PfSPZ invasion is

reported as the percentage reduction in the numbers of PfSPZ that invaded a human hepatocyte line (HC-04) in the presence of post immunisation vs pre-immunisation sera from the same subject, both at a dilution of 1:5. (C) Antibodies to PfCSP by ELISA reported as the difference in OD 1–0 between post-immunisation and pre-immunisation sera (net OD 1·0). (D) Antibodies to PfCSP by ELISA reported as the ratio of post-immunisation OD 1·0 to the pre-immunisation OD 1·0. Ratio of post-immunisation OD 1·0 to pre-immunisation OD 1·0 in the (E) placebo group and (F) PfSPZ Vaccine group, by time to first infection or not having an infection. In each part of the figure, two participants from the vaccine group are not represented because of early withdrawal, and one participant from the placebo group is not represented because of missing samples. PfSPZ=*Plasmodium falciparum* sporozite. PfCSP=*Plasmodium falciparum* circumsporozoite protein. AFU=arbitrary fluorescence units. OD=optical density.

Table 1:

Baseline demographics of participants who received at least one vaccination

	Pilot safety cohort (PfSPZ Vaccine; n=12)	Main cohort	
		PfSPZ Vaccine (n=46)	Placebo (n=47)
Sex			
Male	11 (92%)	38 (83%)	41 (87%)
Female	1 (8%)	8 (17%)	6 (13%)
Age, years			
Mean (SD)	24 (4)	24 (5)	24 (5)
Range	18–32	18–34	18–35
Weight, kg			
Mean (SD)	58 (8)	61 (7)	62 (8)
Range	35–67	50–75	45–79
Village			
Donéguébougou	12 (100%)	15 (33%)	17 (36%)
Banambani	0	11 (24%)	12 (26%)
Torodo	0	15 (33%)	15 (32%)
Zorokoro	0	5 (11%)	3 (6%)
Patent parasitaemia at screening			
Plasmodium falciparum	0	6 (13%)	5 (11%)
Plasmodium malariae	0	1 (2%)	0
Plasmodium ovale	0	0	0
Co-infections *			
Schistosoma haematobium	0	1 (3%)	2 (5%)
Helminth	0	2 (5%)	1 (3%)
Protozoa	1 (11%)	9 (23%)	11 (28%)
Haemoglobinopathies			
Hgb AA	8 (67%)	39 (85%)	38 (81%)
Hgb CC	0	1 (2%)	0
Hgb AC	4 (33%)	6 (13%)	9 (19%)

Data are n (%) unless stated otherwise. PfSPZ=*Plamodium falciparum* sporozite.

* Co-infections were not measured at baseline; they were assessed in 80 participants in the main cohort (40 in the vaccine group and 40 in the placebo group) and all nine participants in the pilot safety cohort who received all five vaccinations on study day 112 (28 days before the fifth vaccination).

Table 2:

Local and systemic adverse events after vaccination

	Pilot safety cohort (PfSPZ Vaccine; n=12)	Main cohort	
		PfSPZ Vaccine (n=46)	Placebo (n=47)
Local symptoms			
Pain or tenderness			
Grade 1	0	0	4 (9%)
Swelling or redness or induration			
Grade 1	0	0	0
Any local symptom			
Grade 1	0	0	4 (9%)
Systemic symptoms			
Fever or feverish			
Grade 1	1 (8%)	0	1 (2%)
Nausea			
Grade 1	0	0	0
Diarrhoea			
Grade 1	0	0	0
Headache			
Grade 1	3 (25%)	3 (7%)	4 (9%)
Fatigue			
Grade 1	1 (8%)	0	1 (2%)
Myalgia			
Grade 1	0	1 (2%)	1 (2%)
Urticaria			
Grade 1	0	0	0
Any systemic symptom			
Grade 1	3 (25%)	3 (7%)	4 (9%)

Data are n (%), where n represents the number of unique participants with the event. No grade 2–5 adverse events were reported. Solicited adverse events were documented for 7 days after each vaccination. Each vaccine receipt is counted once at worst severity for any local and systemic parameter. Laboratory adverse events are shown in the appendix (appendix p 23). PfSPZ=*Plamodium falciparum* sporozite.